Systems Chemistry

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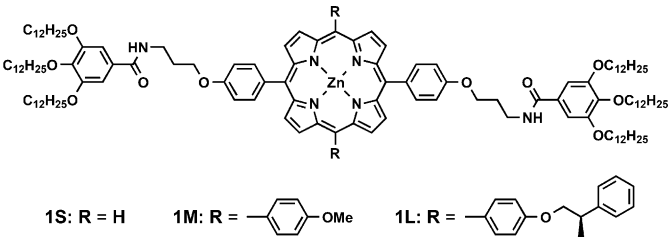
Kinetic Control over Pathway Complexity in Supramolecular Polymerization through Modulating the Energy Landscape by Rational Molecular Design**

Soichiro Ogi, Tomoya Fukui, Melinda L. Jue, Masayuki Takeuchi, and Kazunori Sugiyasu**

Abstract: *Far-from-equilibrium thermodynamic systems that are established as a consequence of coupled equilibria are the origin of the complex behavior of biological systems. Therefore, research in supramolecular chemistry has recently been shifting emphasis from a thermodynamic standpoint to a kinetic one; however, control over the complex kinetic processes is still in its infancy. Herein, we report our attempt to control the time evolution of supramolecular assembly in a process in which the supramolecular assembly transforms from a J-aggregate to an H-aggregate over time. The transformation proceeds through a delicate interplay of these two aggregation pathways. We have succeeded in modulating the energy landscape of the respective aggregates by a rational molecular design. On the basis of this understanding of the energy landscape, programming of the time evolution was achieved through adjusting the balance between the coupled equilibria.*

In nature, far-from-equilibrium thermodynamic systems are commonly found, particularly in biological systems, where they exist as a consequence of the synergistic and reciprocal interplay between a number of equilibria.[1] These systems adapt to environments and function as the occasion demands. It has thus been hypothesized that the realization of such systems by using artificial molecules could lead to autonomous materials. In this context, systems chemistry is an emerging field of research,[2] and pathway complexity in supramolecular aggregates has increasingly attracted attention.[3] We have recently reported a relevant system based on the supramolecular polymerization of a porphyrin molecule (**1M**, Scheme 1), in which two aggregation pathways interplayed.[4]



Scheme 1. Structures of **1S**, **1M**, and **1L** used in this study.

Cooling a hot solution of **1M** in methylcyclohexane (MCH) resulted in the formation of a J-aggregate nanoparticle (Figure 1b, step 1). This J-aggregate was found to be a kinetically formed product, and after a lag time, eventually transformed into the thermodynamically stable one-dimensional H-aggregate, namely, a supramolecular polymer (Figure 1b, step 2). The nonlinear kinetics suggested that the J-to-H-aggregate transformation is an autocatalytic process. This time evolution could be characterized by the t_{50} value, that is, the time at which the transformation is 50% complete.[5] It was found that the higher the initial concentration of the **1M** J-aggregate, the longer the t_{50} value, which suggested that the J-aggregate was the off-pathway intermediate in respect to the supramolecular polymerization on-pathway.[3a,4,5] As shown in Figure 1b, the J- and H-aggregate formation processes exhibited sigmoidal (step 1) and nonsigmoidal (step 3) curves, respectively, which suggested that the former could be described by the isodesmic (or equal-*K*) model, whereas the latter could be described by the cooperative (nucleation-elongation) model.[6–8] Important in this pathway complexity was the fact that the on-pathway supramolecular polymerization (H-aggregate formation) was coupled with the off-pathway pre-equilibrium (J-aggregate formation), thereby creating a kinetic trap. Consequently, the off-pathway kinetically controlled the supramolecular polymerization, which led to the first reported living supramolecular polymerization.[4,7a,b] This pathway complexity of **1M** was unexpectedly found; herein, we determine whether the intricate kinetic process can be rationally controlled by molecular design.

As shown in Scheme 1, we altered the structure of **1M** slightly with respect to the steric hindrance by replacing the

[*] Dr. S. Ogi, T. Fukui, M. L. Jue, Prof. Dr. M. Takeuchi, Dr. K. Sugiyasu
Organic Materials Group, Polymer Materials Unit
National Institute for Materials Science (NIMS)
1-2-1 Sengen, Tsukuba, Ibaraki 305-0047 (Japan)
E-mail: TAKEUCHI.Masayuki@nims.go.jp
SUGIYASU.Kazunori@nims.go.jp
T. Fukui, Prof. Dr. M. Takeuchi
Department of Materials Science and Engineering
Graduate School of Pure and Applied Sciences
University of Tsukuba
1-1-1 Tennodai, Tsukuba, Ibaraki 305-8577 (Japan)

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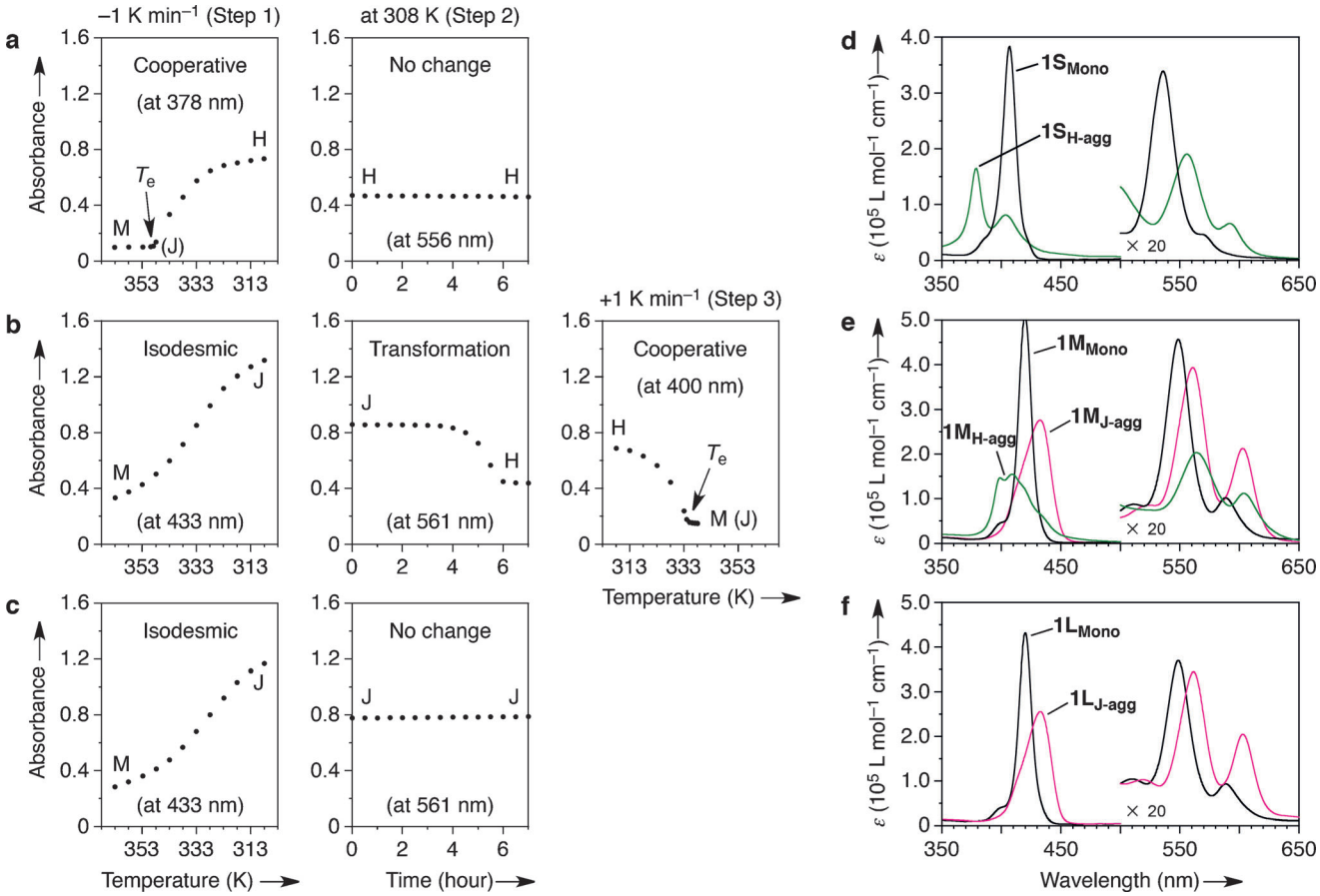

Figure 1. a–c) Plots showing changes in the absorbance at the given wavelengths of a) **1S**, b) **1M**, and c) **1L**, observed during (step 1) cooling, (step 2) incubation, and (step 3) heating processes: [**1S**, **1M**, or **1L**] = 50 μM in MCH; path length of the cuvette for steps 1 and 3 was 1 mm, and that for step 2 was 10 mm; solution was stirred at 400 rpm during step 2. d–f) Absorption spectra of d) **1S**, e) **1M**, and f) **1L** in monomeric (black lines), J-aggregate (pink lines), and H-aggregate (green lines) forms. Sigmoidal and nonsigmoidal curves of the temperature-dependent absorption spectral changes (in steps 1 and 3) were analyzed according to isodesmic and cooperative models, respectively (see the Supporting Information).

medium-sized 4-methoxyphenyl substituents with smaller hydrogen atoms in **1S** and with larger chiral 4-[(*R*)-(+)-2-phenyl-1-propoxy]phenyl groups in **1L**. The synthesis and characterization of these new compounds are described in the Supporting Information. We envisaged that the experiments based on the simplest structural parameter (i.e. steric hindrance) would provide a primary clue to tackle the intricate system.

Similar to the case of **1M**, the self-assembling processes of **1S** and **1L** were investigated by measuring the changes in the absorption spectra as a function of temperature and time (Figure 1 a,c). According to exciton coupling theory, the J- and H-aggregates are characterized by red- and blue-shifted absorption maxima, respectively, compared with those of the corresponding monomeric porphyrin molecules (Figure 1 d,f). Upon cooling of a hot solution of **1S** in MCH, **1S** showed evidence of the formation of J-aggregates (i.e. the appearance of a shoulder at 422 nm; see Figure S1 in the Supporting Information); however, it abruptly underwent the nucleation-elongation process to give the H-aggregate, as characterized by the blue-shifted absorption band and critical temperature (T_e; Figure 1 a, step 1). The transformation to the H-aggregate was also confirmed by the temperature-dependent changes in the fluorescence spectrum, as the H-aggregate is less fluorescent than both the monomeric and J-aggregate forms (see Figure S2 in the Supporting Information).[9] The AFM image revealed that the H-aggregate of **1S** consisted of a fibrous structure with a unimolecular height (ca. 1 nm), similar to that of **1M** (see Figure S6 in the Supporting Information). **1S** has a higher T_e value, and thus, a higher K_E value than **1M**, which is probably due to the stronger π-π stacking of the sterically less-hindered porphyrin plane. Accordingly, for **1S**, the kinetic trap is not deep enough, and therefore, the H-aggregate prevails over the J-aggregate. We note that similar "biphasic" self-assembly pathways have recently been reported by Würthner, Lochbrunner and co-workers.[9] On the other hand, cooling a hot MCH solution of **1L** resulted in it forming a J-aggregate nanoparticle (Figure 1 c, step 1; see also Figures S5 and S7 in the Supporting Information); however, the **1L** J-aggregate did not transform into the H-aggregate at the given temperature and time ranges (step 2). We infer that the bulky substituents in **1L** prevented the porphyrin molecules from stacking in a face-to-face fashion and this destabilized the H-aggregate. We applied isodesmic

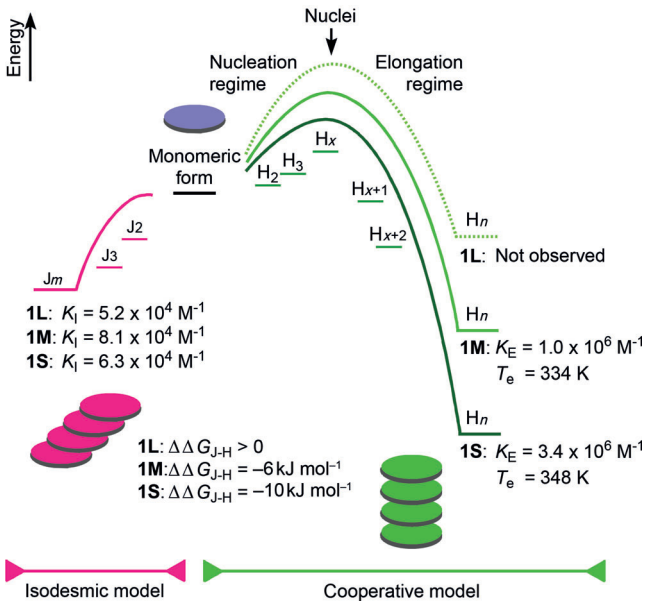

Figure 2. Energy landscape representing the pathway complexity in the supramolecular polymerization of **1S**, **1M**, and **1L**, illustrated on the basis of the thermodynamic parameters determined by van't Hoff plots (see the Supporting Information): $\Delta\Delta G_{J\text{-}H}$ is the free energy difference between the respective J- and H-aggregates; K_Is and K_Es are the aggregation constants for the isodesmic and elongation processes, respectively, at 308 K; T_es are critical temperatures for 50 μM solutions. Note that nuclei sizes "*x*" are not necessarily the same for **1S**, **1M**, and **1L**.

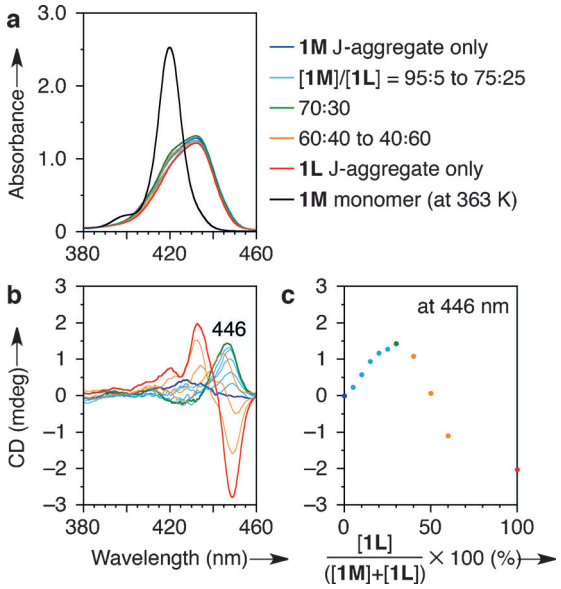

Figure 3. a) Absorption and b) CD spectra of the **1M**/**1L** mixtures. c) Plot of the CD intensity at 446 nm as a function of the **1M**/**1L** ratio. [**1M**] + [**1L**] = 50 μM.

and cooperative models to the temperature-dependent absorption spectral changes of the J- and H-aggregates, respectively, and obtained thermodynamic parameters (see the Supporting Information). As summarized in Figure 2, the K_I values for the J-aggregates of **1S**, **1M**, and **1L** were almost identical, but the K_E value for the H-aggregate of **1S** was more than three times greater than that of **1M**. Accordingly, the steric hindrance at the 5- and 15-positions of the porphyrin molecules mainly affects the stability of the H-aggregate, as it requires more π-surface overlap than the J-aggregate. These results indicate that the energy landscape of the pathway complexity can be rationally modulated by molecular design.

To establish a supramolecular system based on the pathway complexity, we investigated the coassembly of **1M** and **1L**, because both porphyrins can form J-aggregates at the outset. We measured the CD spectra of the **1M**/**1L** mixtures, with the expectation that the (*R*)-(+)-2-phenyl-1-propoxy groups in **1L** would act as a chiral probe. The pure **1L** J-aggregate showed a negative cotton effect, whilst that of **1M** was CD silent (Figure 3b). When a small amount of chiral **1L** was "doped" into the **1M** J-aggregate, an opposite CD signal (positive cotton effect) was observed, while the absorption spectrum of the J-aggregate was still retained (Figure 3a,b). This result implied the formation of a new chiral assembly (see Figure S8 in the Supporting Information for AFM images). The positive CD signal of the **1M**/**1L** mixture intensified, with an accompanying isosbestic point (436 nm), upon increasing the **1L** content up to 30 mol%. Further addition of **1L** induced a deviation from the isosbestic point and inversion of the CD signal (Figure 3c). The isodesmic sigmoidal curves of the **1M**/**1L** coaggregates were independent of the mixing ratio (see Figure S9 in the Supporting Information), thus indicating that the thermodynamic stabilities of the J-aggregates were not significantly influenced. Although the precise molecular packing mode is not clear at present, these results indicate that **1M** and **1L** coassemble into a chiral J-aggregate, as long as the **1L** content is lower than 30 mol%.[10] Interestingly, when we prepared solutions of the **1M** and **1L** J-aggregates individually and then mixed them at 308 K ([**1M**]/[**1L**] = 70:30), the negative cotton effect of the **1L** homoassembly was inverted to the positive signal of the **1M**/**1L** coassembly within 10 min (see Figures S10 and S11 in the Supporting Information), which indicates that reshuffling of the **1M**/**1L** J-aggregate occurs rapidly.

To verify whether the pathway complexity can be controlled on the basis of the above consideration, we evaluated the t_{50} values of the J-to-H-aggregate transformation of **1M** in the presence of **1L**. We fixed the total concentration of the porphyrin molecules (i.e. [**1M** + **1L**] = 50 μM) but changed their mixing ratio; therefore, the actual **1M** concentration was decreased on the addition of **1L**. Interestingly, in the presence of **1L**, the t_{50} value became longer on decreasing the **1M** concentration, a tendency opposite to that observed in the absence of **1L** (Figure 4a, comparing ii and v).[11] The plateaued absorbance changes ($A_{t=0}-A_t$) were dependent on the **1M** concentrations, which suggested that **1L** was not involved in the on-pathway for H-aggregate formation. The AFM image indeed showed the formation of one-dimensional fibers and nanoparticles from the **1M**/**1L** mixture, which were attributable to **1M** H-aggregate and **1L** J-aggregate, respectively (see Figure S12 in the Supporting Information). As such, **1L** indirectly and kinetically influences the pathway complexity of **1M**.

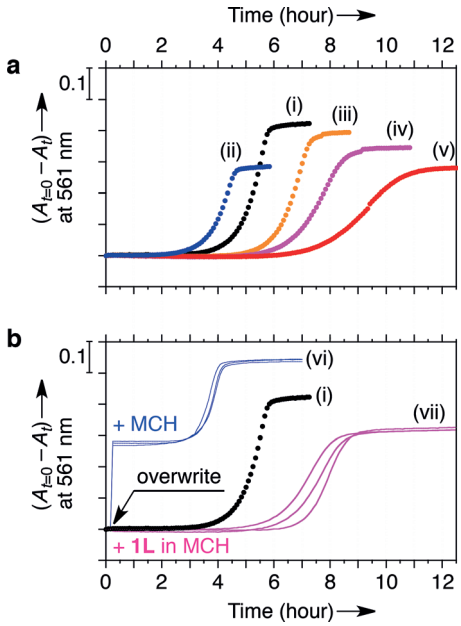

Figure 4. a) Time profile of transformation from J- to H-aggregates: i) [**1M**] = 50 μM, ii) [**1M**] = 35 μM, iii) [**1M**] = 45 μM, [**1L**] = 5 μM, iv) [**1M**] = 40 μM, [**1L**] = 10 μM, and v) [**1M**] = 35 μM, [**1L**] = 15 μM. b) Designated t_{50} value of conditions (i) and (vi) was advanced and (vii) delayed by the addition of pure MCH and **1L** solution, respectively, 10 min after the measurement was started. Each overwriting experiment was reproduced three times. The final concentrations of the porphyrin molecules were (vi) [**1M**] = 35 μM, and (vii) [**1M**] = 40 μM, [**1L**] = 10 μM. During the measurements, the solution was stirred at 400 rpm at 308 K.

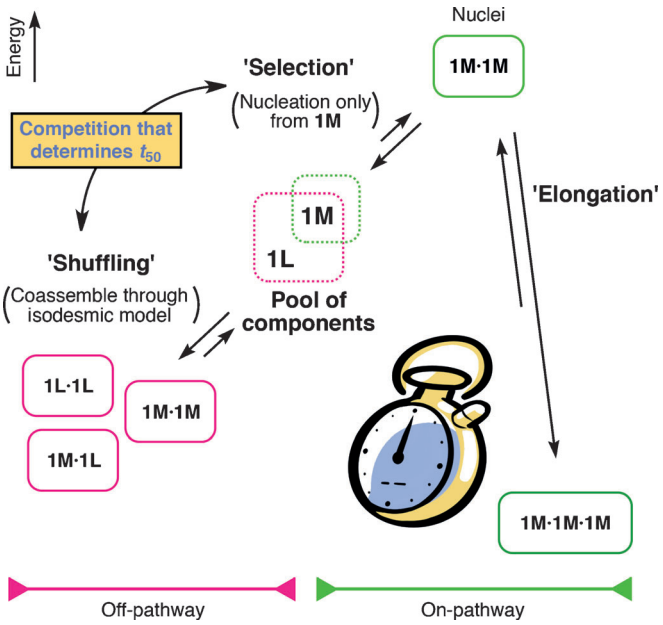

Figure 5. Supramolecular system constructed as a consequence of the coupled equilibria. Compare with Figure 2; here, the simplest situation of $x = 2$, $m = 2$, and $n = 3$ is illustrated for clarity.

As mentioned above, both **1M** and **1L** form J-aggregates, which reshuffle and coassemble when mixed (as long as the content of **1L** is below 30%). Meanwhile, **1M** eventually forms thermodynamically stable H-aggregates through the nucleation step. Hence, the mixture of **1M** and **1L** undergoes either pathway a) "shuffling": the formation of the **1M/1L** J-aggregate coassembly, or b) "selection": the nucleation of **1M** from the pool of **1M/1L** mixture (Figure 5). These two pathways compete and interplay, and the balance between them determines the t_{50} value. This is to say that the algorithm behind the complexity progresses such that the presence of **1L** creates a new pathway to the coassembly, which decreases the fraction of the monomeric **1M**, and thereby retards its nucleation. Accordingly, the time evolution of the transformation is programmable through the concentration of **1M** or the **1M/1L** mixing ratio. We note that such a kinetic perturbation by additives is relevant to the design principle of those artificial peptides that antagonize protein aggregation and reduce amyloid toxicity.[13]

Interestingly, the time programming can even be overwritten, as the reshuffling of the off-pathway J-aggregate formation occurs rapidly and reaches its pre-equilibrium within a few minutes (see above and Figure S10 in the Supporting Information). As shown in Figure 4b, the designated t_{50} value of the J-to-H-aggregate transformation of **1M** could be both advanced by dilution with pure MCH, and delayed by the addition of **1L** solution. This process is similar to the phenomenon called "dilution-induced self-assembly", which is also achieved as a consequence of coupled equilibria.[3c] We assert, therefore, that not only is the pathway complexity important for synthesizing metastable materials that are thermodynamically inaccessible,[14] but it also allows the design of sophisticated materials by taking the time programming into account.

In conclusion, we have studied the pathway complexity in the supramolecular polymerization of porphyrin derivatives based on isodesmic and cooperative models. The energy landscapes of the pathway complexity were modulated by a subtle difference in the steric hindrance. Such a rational control over the pathway complexity can dictate the spontaneous process to be controlled, which should be an important technique for "controlled" supramolecular polymerization.[4] Furthermore, the proof-of-concept experiments demonstrated that the time evolution of the pathway complexity is programmable. We believe that our findings could be a primitive step forward toward constructing far-from-equilibrium thermodynamic supramolecular systems de novo. If such a pathway complexity is incorporated into a non-equilibrium open system, then one could expect the emergence of autonomous materials that function as the occasion demands, much like biological systems.

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Keywords: pathway complexity · porphyrins · supramolecular polymerization · systems chemistry · time evolution

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[11] The t_{50} value for **1M** (50 μM) was 205 min in our previous paper,[4] while the value obtained in this study was 320 min. At present, we attribute the inconsistency of these values to the difference in the production of the MCH used, as the nucleation process is known to be very sensitive to a tiny amount of impurity.[12] We will report this delicate kinetic behavior in due course. The thermodynamic parameters of the **1M** H-aggregate such as ΔH, ΔS, K_e, and T_e values were not affected. In addition, the AFM image of the **1M** H-aggregate taken in this study was identical to our previous result (see Figure S13 in the Supporting Information).

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